IJPSR (2020), Volume 11, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 September 2019; received in revised form, 10 January 2020; accepted, 22 January 2020; published 01 September 2020

IN-VIVO AND STABILITY STUDIES OF DRY POWDER INSUFFLATION CONTAINING TERBUTALINE SULPHATE AND ITRACONAZOLE NANOPARTICLES FOR THE TREATMENT OF ASTHMA

Venugopalaiah Penabaka [1], B. Kumar [*2] and N. B. L. Prasad [3]

Jawaharlal Nehru Technological University [1], Anantapur (JNTUA), Anantapuramu - 515002, Andhra Pradesh, India.
Department of Pharmaceutics [2], Ratnam Institute of Pharmacy, Pidathapolur, Muthukur, SPSR Nellore - 524346, Andhra Pradesh, India.
JNTUA-Oil Technological Pharmaceutical Research Institute [3], Anantapuramu - 515001, Andhra Pradesh, India.

Keywords:

Asthma, Dry powder insufflation, Terbutaline sulphate, Itraconazole, Pharmacokinetics, Stability

Correspondence to Author:
Dr. B. Kumar

Professor and HOD,
Department of Pharmaceutics,
Ratnam Institute of Pharmacy,
Pidathapolur, Muthukur, SPSR Nellore - 524346, Andhra Pradesh, India.

E-mail: drkumarpharma1973@gmail.com

ABSTRACT: The present research was envisaged on the development of dry powder to treat asthma. Terbutaline sulphate (a bronchodilator) and Itraconazole (an antifungal) were used in the present study for bronchodilation and allergy to *Aspergillus fumigatus* (fungi) using lactose and trehalose as excipients. Dry powder insufflations were prepared by physical mixing (milling) and spray drying, out of which spray dried formulations with lactose as excipient gave the best results *in-vitro*. Hence, spray dried formulations were preceded for further pharmacokinetic and stability studies. The pulmonary concentrations of Terbutaline sulphate and Itraconazole from TER – A (sd), ITR – A (sd), TER: ITR – A (sd) monotonically decreased (T_{max} = 0 min). However, Itraconazole showed higher AUC0-α in individual and combined formulation when compared to Terbutaline sulphate showing slower elimination of Itraconazole. From plasma pharmacokinetic data T_{max} of Itraconazole formulations ITR – A (sd), TER: ITR – A (sd) was high when compared to the formulations of Terbutaline sulphate TER – A (sd), TER: ITR – A (sd) showing lower systemic bioavailability of Itraconazole when compared to Terbutaline sulphate. Stability studies for Drug content and *in-vitro* dispersion performance were conducted and results showed a decrease in drug content when kept at 40 ± 2 ºC and 75% ± 5% RH. The MMAD and GSD values were increased (from 4.67 µm to 6.32 µm whereas the GSD values were 2.24-3.48 µm) on stress conditions during accelerated, intermediate and long term stability studies.

INTRODUCTION: Invasive pulmonary aspergillosis is a disease caused by *Aspergillus fumigates* (fungi) which is one of the main reasons for asthma with high morbidity and mortality (up to 90%) [1, 2]. Hence, Itraconazole (ITR) is used as a first-line drug to treat aspergillosis [3]. Terbutaline sulphate (TER) is used for bronchodilation as a supportive treatment for the above infection. The concentration of Itraconazole in lung should be high at the alveolar region to inhibit the growth of Aspergillus with low plasma concentration [4].

Hence, dry powder insufflation (DPI) was found to be the best method to target the drug directly to the lungs. Itraconazole is a poorly water-soluble drug which is a major obstacle to formulate it as DPI. The undissolved ITR may be eliminated by macrophages resulting in reduced drug concentration in the lung [5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(9).4550-57

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(9).4550-57

Hence, in the present research, ITR, TER, and excipients were jet-milled to improve the effective surface area of the particle improving bioavailability. Too fast dissolution may show negative effects which result in higher plasma concentrations with low alveolar concentration. Hence to improve bioavailability with high dose delivery to lungs besides improving chemical stability, Dry powder insufflation of Terbutaline sulpahte and Itraconazole was developed using lactose and trehalose as carriers and evaluated for *in-vivo* and stability studies.

MATERIALS AND METHODS:

Materials: Terbutaline sulphate and Itraconazole was obtained from KP Labs Hyderabad. Lactose was obtained from Drugs India Hyderabad. Trehalose from Hayashibara Co. Ltd. Japan. Ethyl carbonate (Urethane), Sodium dihydrogen phosphate dehydrate, Hexane, Dichloromethane was obtained from Himalaya Scientific, Nellore and all the other chemicals used were of analytical grade.

Methods:

Formulation Method – Optimized Formulations: Spray drying process using dilute solutions was performed in a mini spray dryer (SS Laboratory Spray Dryer (LSD 01)) using high-performance cyclone separator and all the ingredients were taken in specified quantities shown in **Table 1**.

TABLE 1: FORMULATION TABLE – SPRAY DRYING

Method	Formulation no.	Formulation code	Drug: Carrier (Milled)	TPC (% W/V)	Solvent used
Spray drying	1	TER – A (sd)	Terbutaline: Lactose	0.4	Methanol
	2	ITR – A (sd)	Itraconazole: Lactose	0.4	Methanol
	3	TER:ITR – A (sd)	Terbutaline: Itraconazole: Lactose	0.8	Methanol

**TPC – Total Powder Concentration

TABLE 2: OUTLET TEMPERATURES OF SPRAY DRIED FORMULATIONS

Spray Drying	Molar Ratios	Outlet temperature ºC
Terbutaline-Lactose	1:1	66
Itraconazole-Lactose	1:1	58
Terbutaline-Itraconazole-Lactose	1:1:1	61

Spray Drying-Conditions:

Automization Rate: 600 L/h
Pump Rate: 15 ml/min (Medium Pump Rate)
Inlet Temperature: 150 ºC
Nozzle Diameter: 0.7 mm

Spray died particles were separated by cyclone separator from the nitrogen drying air and sealed in the glass vials and stored in dessicator under ambient pressure.

***In-vivo* studies:**

Pharmacokinetic Parameters of Terbutaline Sulphate and Itraconazole in Lung and Plasma after Single Dose of Insufflation: Six to eight week old Wistar rats weighing about 200-250 g were purchased from National Institute of Nutrition, Hyderabad. Rats were housed 5 per cage under a condition of 23 ± 2 ºC with 55 ± 5% relative humidity and 12 h light/dark cycle. Food and water were provided *ad libitum* until the day before the experiment. Rats fasted for 24 h before dosing. All the experiments and protocols were approved by the Animal Ethical and Welfare Committee at Mahathi College of Pharmacy, Madanapalli, Andhra Pradesh. (Approval number: 1952/PO/Re/S/17/CPCSEA/103/27/04/2019) and with permission from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Pharmacokinetic Studies in Wistar Rats: Human lung volume (4.341 L) was used to calculate the human dose of Terbutaline sulpahte and Itraconazole for pulmonary administration. Equivalent dose calculation for rat has been carried out from the human dose to maintain the plasma concentration above minimum inhibitory concentration for a period of 24 h.

Nasal insufflator was obtained to administer the drug to the pulmonary route, but this device is suitable for human use and not for animal administration. So, a dry powder inhaler delivery device was designed with little modification. Wistar rats were divided into three groups (Ten rats in each group) and anesthetized by intraperitoneal injection of 20% urethane (5 ml/kg). Gently pulled

the tongue outside and sprayed DPIs through the device by placing it in trachea region [6, 7]. DPI formulations were insufflated by exhausting 1.5 ml of air which corresponds to the tidal volume of Wistar rats at a relatively stable speed. Insufflators was weighed before and after powder filling and after administration to determine the actual amount of sample emitted and the amount present in the lung. The polyethylene tubing was cannulated to the right jugular vein for blood sampling. At least 3 ml of blood was collected in pre-heparinized tubes and then centrifuged at 4500 rpm for 15 min. At a predetermined time intervals (5 min, 0.5 h, 1 h, 2 hrs, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h) Wistar rats were humanely sacrificed and the whole lung without the trachea and visible bronchi were excised by surgical resection. Lung samples were weighed and stored at -20 ºC until analysis.

Group 1: Rats were administered a single intratracheal dose (0.5 mg/kg) of formulation (TER-A (SD)) [8].

Group 2: Rats were administered a single intratracheal dose (8.5 mg/kg) of Itraconazole (ITR-A (SD)) [9].

Group 3: Rats were administered a single intratracheal dose of formulation (TER: ITR-A (SD)).

The concentration of Terbutaline sulphate and Itraconazole was determined by UV/Vis-spectroscopy (λ_{max}: 276 nm for Terbutaline, λ_{max}: 264 nm for Itraconazole). A calibration curve within the range of 10 – 100 µg/ml (R^2 = 0.99 was established for analysis).

Assay: For assay of Terbutaline sulphate and Itraconazole in pharmacokinetic studies, samples were processed by adding a predetermined volume of sodium chloride solution to the harvested lungs at 5:1 v/w for homogenization. Then 250 µL of lung tissue homogenate was extracted. 80 µL of the $ZnSO_4$ solution was then added for protein precipitation.

The obtained suspension was extracted with 5 ml of hexane-dichloromethane (70:30 v/v). After centrifugation at 10000 rpm for 15 min, the upper organic layer was collected. Extraction was done twice and the organic layers were added and dried in a vacuum oven. The obtained residue was reconstituted and filtered (0.22 µm) for assay [10].

Pharmacokinetic Analysis: Pharmacokinetics is concerned with the variation in drug concentration with time as a result of absorption, distribution and elimination [11, 12]. The time course of drug action depends on:

A. Drug dose, route of administration, rate, and extent of absorption, distribution rate (particularly to the site of action) and rate of elimination.

B. The minimum effective concentration and concentration-effect relationship.

T_{max} and C_{max}: The peak time (T_{max}) and the peak concentration (C_{max}) may be directly obtained from the experimental observations of each animal.

Half-life: The time required to reduce the plasma concentration to one half its initial value is defined as the half-life ($t_{1/2}$).

$$t_{1/2} = 0.693 / K_e$$

The Area Under the Curve (AUC): The under the curve (AUC) is a parameter that may be used in different ways depending on the experimental context. In the present research, AUC was calculated using the trapezoidal rule. This parameter may be used as an index of the drug exposure of the body, when referred to the plasma drug levels, or as an index of the drug exposure of particular tissues if referred to the drug levels in tissues. Under very general assumptions, the area under the plasma or blood drug concentrations is a parameter that is closely dependent on the drug amount that enters into the systemic circulation and on the ability that the system has to eliminate the drug (clearance). Therefore, it can be used to measure the drug amount absorbed or the efficiency of physiological processes that characterize drug elimination.

Volume of Distribution: The volume of distribution (V_d) has no direct physiological meaning; it is not a 'real' volume and is usually referred to as the apparent volume of distribution. It is defined as the volume of plasma in which the total amount of drug in the body would be required to be dissolved in order to reflect the drug concentration attained in plasma.

$$V_d = X / C$$

X = Amount of drug, C = Concentration of drug in plasma

Clearance: Drug clearance (CL) is defined as the volume of plasma in the vascular compartment cleared of drug per unit time by the processes of metabolism and excretion. Clearance for a drug is constant if the drug is eliminated by first-order kinetics. The drug can be cleared by renal excretion or by metabolism or both. With respect to the kidney and liver, *etc*., clearances are additive, *i.e.*

$$CL_{total} = CL_{renal} + CL_{nonrenal}$$

Mathematically, clearance is the product of the first-order elimination rate constant (k) and the apparent volume of distribution (V_d). Thus,

$$CL_{total} = k \times V_d$$

Clearance is related to half-life by

$$t_{1/2} = (0\ 693 \times V_d) / CL$$

(TER-A (SD)), (ITR-A (SD)) and (TER: ITR-A (SD)) concentrations in the lung tissue/plasma were plotted versus time and then analyzed for non-compartmental analysis. Pharmacokinetic profiles were characterized by half-life, maximum concentration (C_{max}), maximum time (T_{max}), area under the curve (AUC 0-24 h), Mean residence time (MRT 0-24 h). The results were shown in **Table 5** and **Fig. 1-4**.

Stability Studies: Stability studies were conducted for 6 months (Batch-1 Accelerated Stability Studies at 40 ± 2 ºC and 75% ± 5% RH), (Batch -2 Intermediate Stability Studies at 30 ± 2 ºC and 65% ± 5% RH), for 12 months (Batch-3 Long Term stability studies at 25 ± 2 ºC and 60% ± 5% RH) [13].

Assay (Drug Content Determination): Transferred 10 capsules into a 100 ml volumetric flask (Terbutaline dose equivalent to 50 mg and Itraconazole dose equivalent to 500 mg) and sonicated to dissolve the capsule by adding the suitable volume of diluent (Water for Terbutaline sulphate and Methanol for Itraconazole), for about 10 min with intermittent shaking (for complete dispersion) and made the volume with diluent. Filtered through a 0.45 µ membrane and estimated Terbutaline Sulphate at 276 nm and Itraconazole at 264 nm using UV spectrophotometer (2060 Plus UV-VIS Dual Beam, Analytical Technologies Pvt. Ltd.).

Determination of MMAD using Cascade Impactor: Mass median aerodynamic diameter (MMAD) represents aerodynamic diameter below which 50% particles remain. Aerodynamic diameter of a particle controls its deposition in the pulmonary tract. MMAD of the optimized dry powder inhaler was determined using a seven-stage cascade impactor. Firstly, the capsules were needle pierced and were drawn into the cascade impactor with a controlled flow rate of 28.3 L/min with a delay time of 10 s. This was done with a total of 3 capsules per sample for a total of 25.5 mg (TER-A) total per run, 100 mg (ITR-A) total per run and 125.5 mg (TER: ITR-A) total per run. After deposition of DPI, Terbutaline and Itraconazole content was determined in each chamber by UV Spectroscopy. Absorbance was detected at 276 nm for Terbutaline and 264 nm for Itraconazole. The estimated drug content in each chamber was inserted into the MMAD Calculator to obtain MMAD and geometric standard deviation (GSD). Other parameters like fine particle fraction, extra-fine particles were determined [14].

RESULTS:

TABLE 3: PHARMACOKINETIC PROFILES OF TERBUTALINE SULPHATE AND ITRACONAZOLE IN LUNG TISSUE

Time (Min)	Conc (µg/g of Lung)			
	TER-A (SD)	ITR-A (SD)	TER: ITR-A (SD) TER	TER: ITR-A (SD) ITR
5	23.11 ± 1.21	41.32 ± 1.19	22.89 ± 1.44	43.12 ± 1.86
30	16.82 ± 0.89	33.11 ± 1.34	19.17 ± 1.23	36.32 ± 1.20
60	11.31 ± 0.93	25.32 ± 1.23	10.32 ± 1.31	29.34 ± 1.33
120	9.32 ± 0.81	18.17 ± 1.10	8.89 ± 1.24	16.65 ± 1.21
240	7.89 ± 0.81	12.13 ± 1.21	6.35 ± 0.93	9.56 ± 0.78
360	6.92 ± 0.67	10.02 ± 0.93	5.34 ± 0.53	7.33 ± 0.89
480	5.12 ± 0.68	8.92 ± 0.89	4.21 ± 0.55	6.12 ± 0.57
720	3.73 ± 0.56	7.34 ± 0.78	3.33 ± 0.43	4.87 ± 0.65
960	2.11 ± 0.48	5.12 ± 0.77	2.01 ± 0.54	3.78 ± 0.41
1440	1.89 ± 0.36	3.26 ± 0.59	1.55 ± 0.41	3.17 ± 0.43

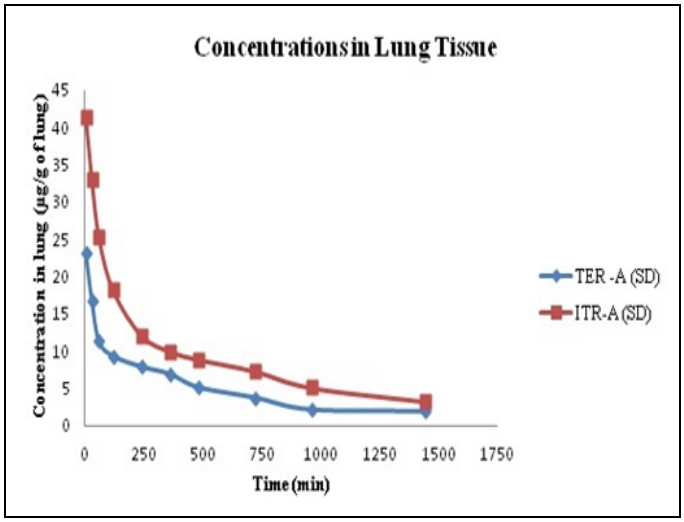

FIG. 1: PHARMACOKINETIC PROFILES OF TERBUTALINE SULPHATE AND ITRACONAZOLE IN LUNG TISSUE

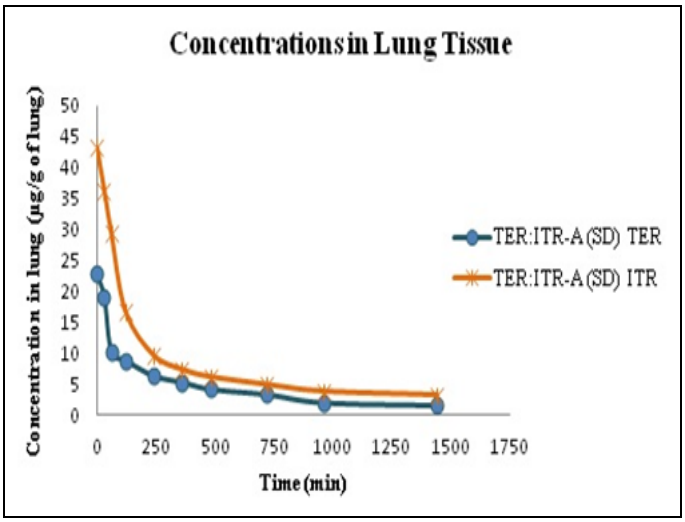

FIG. 2: PHARMACOKINETIC PROFILES OF TERBUTALINE SULPHATE AND ITRACONAZOLE IN LUNG TISSUE (IN COMBINATION)

TABLE 4: PHARMACOKINETIC PROFILES OF TERBUTALINE SULPHATE AND ITRACONAZOLE IN PLASMA

Time (Min)	Conc in Plasma (ng/ml)			
	TER -A (SD)	ITR-A (SD)	TER: ITR-A (SD) TER	TER: ITR-A (SD) ITR
30	53.23 ± 1.89	40.53 ± 1.66	52.78 ± 2.10	42.23 ± 2.26
60	98.34 ± 2.21	104.22 ± 2.34	100.32 ± 2.32	101.34 ± 2.45
120	143.52 ± 2.63	180.23 ± 2.32	141.94 ± 2.51	179.34 ± 2.91
240	80.03 ± 1.94	273.33 ± 2.59	78.83 ± 1.87	260.23 ± 2.78
360	74.23 ± 2.13	123.23 ± 1.87	71.12 ± 1.89	132.23 ± 2.10
480	63.24 ± 1.21	78.45 ± 1.65	62.11 ± 1.34	76.34 ± 1.45
720	51.22 ± 1.13	72.14 ± 1.78	53.32 ± 1.21	74.12 ± 1.73
960	41.02 ± 1.10	64.23 ± 1.63	39.93 ± 1.45	63.34 ± 1.35
1440	24.24 ± 1.33	48.32 ± 1.77	28.64 ± 1.32	45.53 ± 1.32

TABLE 5: PHARMACOKINETIC PARAMETERS OF TERBUTALINE SULPHATE AND ITRACONAZOLE IN LUNG AND PLASMA AFTER SINGLE DOSE OF INSUFFLATION

Parameters	TER -A (SD)		ITR -A (SD)		TER: ITR-A (SD) TER		TER: ITR-A (SD) ITR	
	Lung	Plasma	Lung	Plasma	Lung	Plasma	Lung	Plasma
T_{max} (min)	5.0	120	5.0	240	5.0	120	5.0	240
C_{max} (µg/g for lung, ng/ml for plasma)	23.11 ± 7.45	143.52 ± 98.45	41.32 ± 8.09	273.33 ± 126.32	22.89 ± 6.32	141.94 ± 78.32	43.12 ± 6.56	260.23 ± 132.21
AUC $_{0\text{-}24h}$ (µg.min/g for lung, ng.min/ml for plasma)	6906.37	80912.55	12852.53	135260	6133.20	81299.7	10775.40	133689.2
AUC $_{0\text{-}\alpha}$ (µg.min/g for lung, ng.min/ml for plasma)	6936.85 5	81323.39	12905.11	136870	6153.59	81896.3	10852.71	135112
Ka (hr^{-1})	0.032	0.006	0.022	0.009	0.032	0.006	0.018	0.009
Ke (hr^{-1})	0.062	0.059	0.043	0.030	0.076	0.048	0.041	0.032
MRT	454.94	546.51	467.75	544.00	444.17	559.01	432.08	542.04
$T_{1/2}$ (min)	670.64	704.74	966.97	1386.0	547.10	866.25	1014.10	1299.37

TABLE 6: DRUG CONTENT – (STABILITY STUDIES)

Formulation Code	Batch - 1		Batch - 2		Batch - 3	
Spray Drying	%	%	%	%	%	%
TER – A (sd)	94.5 ± 0.23	---	96.4 ± 0.16	-	95.7 ± 0.36	---
ITR – A (sd)	---	95.8 ± 0.31	---	97.9 ± 0.34	---	97.1 ± 0.48
TER:ITR – A (sd)	95.3 ± 0.13	94.8 ± 0.16	96.3 ± 0.16	95.5 ± 0.11	95.9 ± 0.33	95.1 ± 0.24

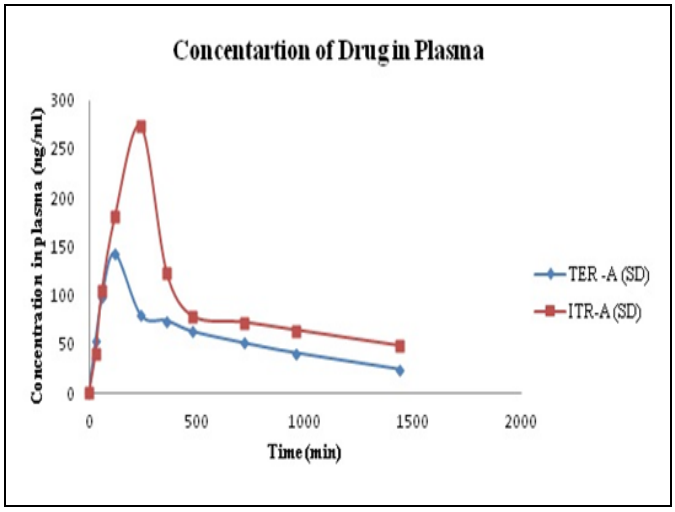

FIG. 3: PHARMACOKINETIC PROFILES OF TERBUTALINE SULPHATE AND ITRACONAZOLE IN PLASMA

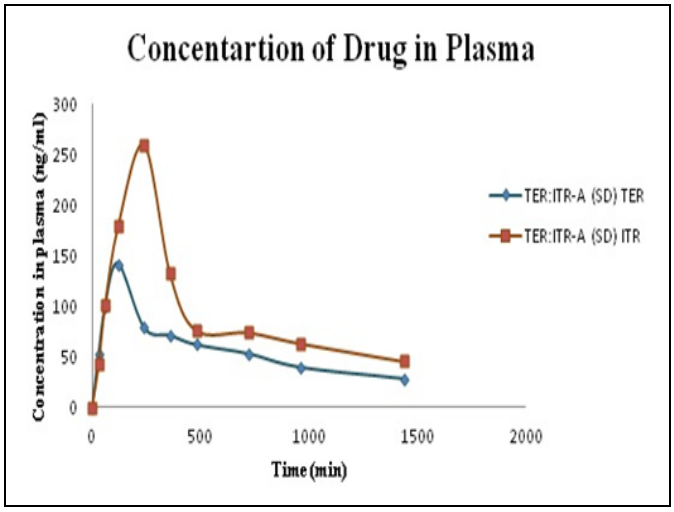

FIG. 4: PHARMACOKINETIC PROFILES OF TERBUTALINE SULPHATE AND ITRACONAZOLE IN PLASMA (IN COMBINATION)

Determination of MMAD using Cascade Impactor – (Stability Studies):

TABLE 7: DETERMINATION OF MMAD USING CASCADE IMPACTOR (BATCH - 1)

Method	Formulations	MMAD (µ)	GSD (µ)	FPF %	RF %	ED %
Spray Drying	TER – A (sd)	5.07	2.96	66.33 ± 0.63	70.01 ± 0.56	94.74 ± 0.34
	ITR – A (sd)	6.32	2.57	61.71 ± 0.45	66.43 ± 0.42	92.90 ± 0.31
	TER:ITR – A (sd)	6.11	3.44	63.69 ± 0.54	66.84 ± 0.31	95.29 ± 0.23

TABLE 8: DETERMINATION OF MMAD USING CASCADE IMPACTOR (BATCH - 2)

Method	Formulations	MMAD (µ)	GSD (µ)	FPF %	RF %	ED %
Spray Drying	TER – A (sd)	4.67	2.51	71.89 ± 0.31	75.20 ± 0.38	95.60 ± 0.43
	ITR – A (sd)	5.55	2.24	66.90 ± 0.45	71.33 ± 0.31	93.80 ± 0.32
	TER:ITR – A (sd)	5.62	3.22	67.75 ± 0.42	70.63 ± 0.21	95.93 ± 0.44

TABLE 9: DETERMINATION OF MMAD USING CASCADE IMPACTOR (BATCH - 3)

Method	Formulations	MMAD (µ)	GSD (µ)	FPF %	RF %	ED %
Spray Drying	TER – A (sd)	4.73	2.64	70.61 ± 0.32	74.01 ± 0.43	95.41 ± 0.34
	ITR – A (sd)	5.86	2.31	65.17 ± 0.21	69.86 ± 0.23	93.30 ± 0.23
	TER:ITR – A (sd)	5.76	3.48	66.52 ± 0.36	69.49 ± 0.26	95.73 ± 0.31

In-vitro aerosol dispersion performance *via* Cascade impactor (mean ± S.D, n = 3) MMAD-Mean median aerodynamic diameter, GSD-Geometric standard deviation, FPF-Fine particle Fraction, RF-Respirable Fraction, ED-Emitted Dose

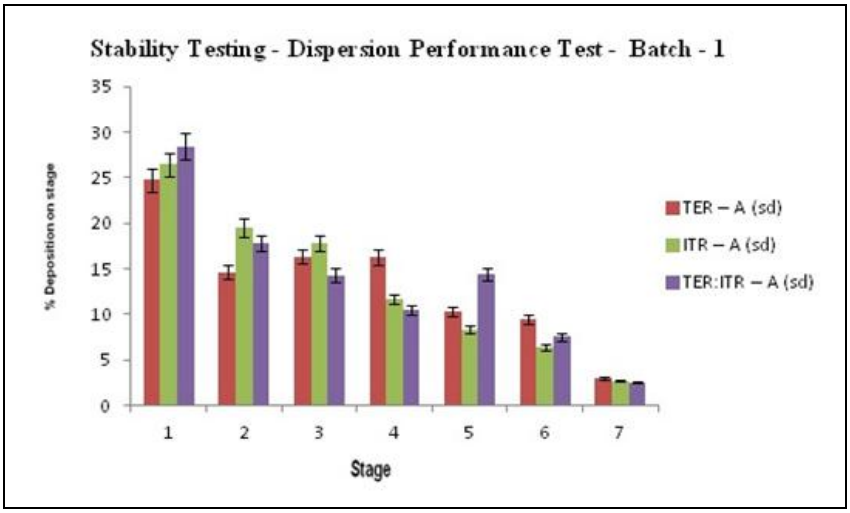

FIG. 5: STABILITY TESTING - DISPERSION PERFORMANCE TEST - BATCH - 1

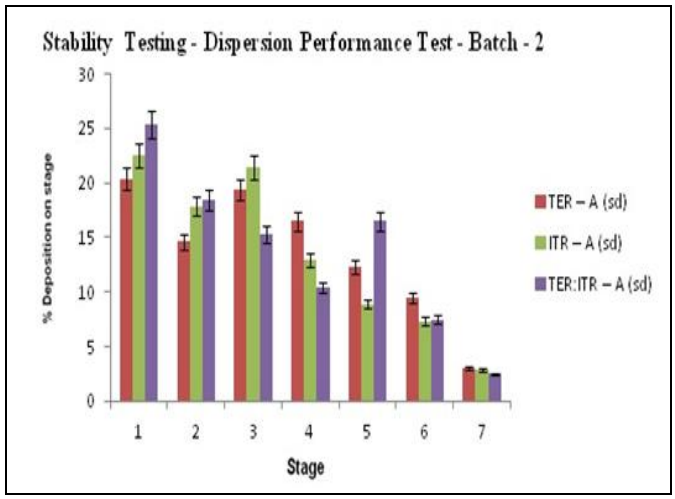

FIG. 6: STABILITY TESTING - DISPERSION PERFORMANCE TEST - BATCH – 2

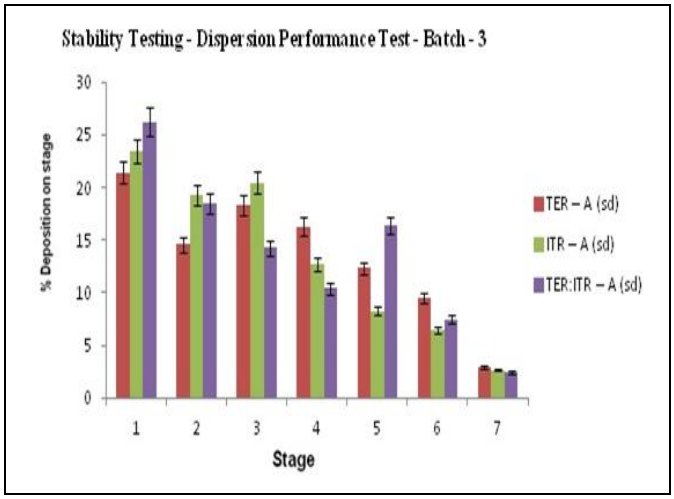

FIG. 7: STABILITY TESTING - DISPERSION PERFORMANCE TEST - BATCH - 3

DISCUSSION: Pharmacokinetic studies were performed to evaluate the *in-vivo* distribution characteristics of TER – A (sd), ITR – A (sd), TER: ITR – A (sd). After insufflation with a specified dose of above formulations, the concentration of Terbutaline sulphate and Itraconazole in lung tissue and plasma were assayed at different time points. The pulmonary pharmacokinetic profiles and parameters of the formulations in the lung are shown in **Fig. 1**, **2** and **Table 3**. The concentrations of Terbutaline sulphate and Itraconazole from TER – A (sd), ITR – A (sd), TER: ITR – A (sd) monotonically decreased (T_{max} = 0 min). Almost same C_{max} values of Terbutaline sulphate was seen in the individual (TER – A (sd)) and combined formulations (TER: ITR – A (sd)), the C_{max} values of Itraconazole was also found to be nearly same in the individual (ITR – A (sd)) and combined formulations (TER: ITR – A (sd)) indicating similar lung deposition.

However, Itraconazole showed higher AUC0-α in individual and combined formulation when compared to Terbutaline sulphate indicating slower elimination of Itraconazole. The plasma pharmacokinetic data of the formulations are shown in **Fig. 3**, **4** and **Table 4**. T_{max} of Terbutaline formulations TER – A (sd), TER: ITR – A (sd) was found to be 120 mts and T_{max} of Itraconazole formulations ITR – A (sd), TER: ITR – An (sd) was found to be 240 mts indicating lower systemic bioavailability of Itraconazole when compared to Terbutaline sulphate. Improvement of the dissolution of the water-insoluble drug (Itraconazole) for pulmonary delivery may help to reduce the elimination of an undissolved drug by macrophages and relieve lung irritation. Fast dissolution may cause fast absorption of the dissolved drug into the plasma and thus short retention in the lung. So, the balance between dissolution enhancement and *in-vivo* distribution is desired for the water-insoluble drug for targeted drug release to the lung. Stability studies were conducted for best formulations (TER – A (sd), ITR – A (sd), TER: ITR – A (sd)) according to ICH guidelines for drug content and *In-vitro* dispersion performance for 12 months as accelerated, intermediate and long term studies.

From the results, it was found that drug content was decreased over 2% within 6 months when the formulations were stored at 40 ± 2 ºC and 75% ± 5% RH (Batch – 1). Drug content decreased partially when the formulations were stored at 30 ± 2 ºC and 65% ± 5% RH, 25 ± 2 ºC and 60% ± 5% RH (Batch – 2 and 3). The decrease in drug content for batch -1 may be due to accelerated stress of high temperature and relative humidity. The results were given in **Table 6**. The aerosol dispersion properties of best formulations were evaluated using the Cascade Impactor coupled with a Rotahaler DPI device. The MMAD values ranged from 3.45 µm to 4.21 µm whereas the GSD values were 1.85-2.83 µm during normal conditions. The MMAD and GSD values were increased (from 4.67 µm to 6.32 µm whereas the GSD values were 2.24-3.48 µm) on stress conditions during accelerated, intermediate and long term stability studies. Aerosol deposition on each stage is measurable and in particular, deposition on the lower stages of

stage 2 all the way to stage 7 (the lowest stage) is observed. The % deposition on stage 1 increased for (Batch-1) when compared to other batches. Due to this, the particles may deposit predominantly in the middle lung regions by sedimentation due to gravitational settling. The results were given in **Table 7-9** and **Fig. 5-7**.

CONCLUSION: The study compared different DPI formulations of Terbutaline sulphate and Itraconazole in single and combined formulation to analyze the *in-vivo* distribution characteristics and stability. Among all the formulations Itraconazole in single and combined formulations showed higher AUC0-α indicating slower elimination of Itraconazole from lung when compared to the formulations containing Terbutaline sulphate. Airway dissolution and systemic absorption should be taken into consideration for developing DPI formulation or poorly soluble drug ITR. Hence we can conclude that with improving dissolution and drug retention in the lung, the above said DPI's can be formulated for the treatment of asthma.

ACKNOWLEDGEMENT: The authors are heartily thankful to the Management for performing to do the work and providing all facilities and chemicals, To Dr. M. Gobinath M. Pharm., Ph.D., Principal, Ratnam Institute of Pharmacy for his constant encouragement to the support and to the Teaching staff for their timely help and support.

CONFLICTS OF INTEREST: The authors declare that no conflicts of interest are associated with this work.

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How to cite this article:
Penabaka V, Kumar B and Prasad NBL: *In-vivo* and stability studies of dry powder insufflation containing terbutaline sulphate and itraconazole nanoparticles for the treatment of asthma. Int J Pharm Sci & Res 2020; 11(9): 4550-57. doi: 10.13040/IJPSR.0975-8232. 11(9).4550-57.